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14. ABSTRACT
The objective of the proposed research is to develop a device to reduce the frequency of breast re-excision surgery in patients with breast malignancies. The purpose of Task 1 was to design a spectral imaging system that will provide two-dimensional measurements approximately 0.5-2.0 mm within breast tissues. Monte Carlo simulations determined that the spacing between each imaging channel should be at least 10mm to have an SNR greater than 100. The monochromator slit size and channel spacing determined that there could be 25, 600um illumination fibers and 25 channels. Four collection fibers per channel was optimal to be certain that all fibers could be imaged on the CCD without any cross-talk between signals. The purpose of Task 2 was to build a fiber optic probe with two imaging channels rather than the entire imaging probe to test the conceptual design. The two-channel probe has been built in our laboratory using 4, 200 μm collection fibers and 1, 600 μm illumination fiber in each channel.

15. SUBJECT TERMS
Margin, cross-talk, spectral imaging system, diffuse reflectance, Monte Carlo, signal-to-noise ratio

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Introduction

In 2007, an estimated 178,480 women in the United States will be diagnosed with invasive breast cancer and another 62,030 will be diagnosed with carcinoma in-situ (CIS) or Stage 0 breast cancer (1). Breast cancer is the second leading cause of cancer-related deaths in women in the U.S. (2). Though regional differences exist, 45-60% of women with early stage invasive breast cancer and/or CIS receive breast conserving surgery (BCS) each year (3). BCS involves removal of malignant tissue with a surrounding margin of normal breast tissue. After pathologic assessment, the results are reviewed and a decision made for a re-excision partial mastectomy versus simple mastectomy if there is evidence of positive or close margins. A positive or close margin is where there are non-invasive or invasive tumor cells within 2.0 mm of the edge of the tissue excised. Currently the rate of re-excision post-BCS performed at NCI (National Cancer Institute) designated cancer centers can vary from 10-40% (4). Thus, there is an unmet clinical need for effective intraoperative assessment of tumor margins in patients with breast cancer.

The objective of the proposed research is to develop a device to reduce the frequency of breast re-excision surgery and the risk of local recurrence in patients with invasive and non-invasive breast malignancy. The purpose of Tasks 1-3 is to develop a multi-channel optical device for intraoperative imaging of each margin in specimens freshly excised from patients undergoing breast conserving surgery. The device will be used to collect diffuse reflectance spectrum in the visible wavelength range from each of multiple pixels. The spectra will be analyzed by an inverse Monte Carlo model developed by our group to extract optical absorption and scattering properties from the spectral data at each pixel which can then be translated into maps of tissue biochemical and structural properties (6, 10). The endogenous absorbers in tissue that can be extracted include oxygenated and deoxygenated hemoglobin, beta carotene, electron carriers and structural proteins. The device can also be used to image exogenous sources of absorption (organic dyes) and scattering (nanoparticles) and thus can provide the concentration and distribution of these agents in tissue. These features will be used to identify positive margins and their location. This information will guide the surgeon to complete the surgery, if margins are negative, or re-excise additional tissue from areas in the cavity corresponding to the specific margins that are positive, thus preventing repeat surgery and the risk of local recurrence.

The purpose of Task 1 was to design a spectral imaging system that will provide two-dimensional measurements approximately 0.5-2.0 mm within breast tissues for detecting close and positive tumor margins in freshly excised partial mastectomy specimens. The purpose of Task 2 was to build a fiber optic probe with two imaging channels rather than the entire 25 channel probe to test the conceptual design. Results from Task 2 will determine if any changes need to be made to the original design. The results of the completed work from Task 1 and Task 2 are presented below.

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Body

In the past year the focus of the project has changed along with the Statement of Work. Included is a revised Statement of Work that reflects the new outcomes of the project. Most research done by our group, as well as other research groups in biomedical optics have focused on designing and building single pixel devices to obtain optical measurements of tissue. Single pixel devices are only capable of providing information about a very small area of tissue. For margin assessment, surgeons who are looking at a larges area and trying to determine if they have excised all positive tissue, the data obtained by a single pixel device does not provide enough information and would require a lot of time to survey the whole margin. Therefore, it is more important to develop a device that is capable of covering more tissue area in a shorter amount of time which is why this Statement of Work is focused on designing, fabricating, and testing a multi-pixel imaging device. Compared to single-pixel devices, multi-pixel devices have the advantage of: significantly increasing the speed for margin surveillance, which is an important physician criterion, providing non-destructive evaluation of up to 80% of the tumor margin, and providing higher resolution (important for focal diseases) and more reproducible measurements.

The objective of Task 1 is to design an optical spectral imaging system that will provide 2-D measurements from a depth of up to 2 mm within breast tissue in order to detect close and positive tumor margins in freshly excised partial mastectomy specimens. Figure 1 shows a schematic of the main components of the system, which consist of a Xenon lamp, a monochromator, a slit, a fiber optic imaging probe, and a CCD camera with imaging optics. White light from the Xenon Lamp passes through the monochromator, and is coupled into the illumination fibers via the illumination fiber adaptor, and propagates to the distal end of the imaging probe. Diffuse reflectance from the tissue is detected by the collection fibers in each channel and propagates back to the collection fiber adaptor, where it is imaged by the CCD camera through imaging optics. Tens to hundreds of single-pixel probes can be built into an imaging array which can be used for 2-D measurements.
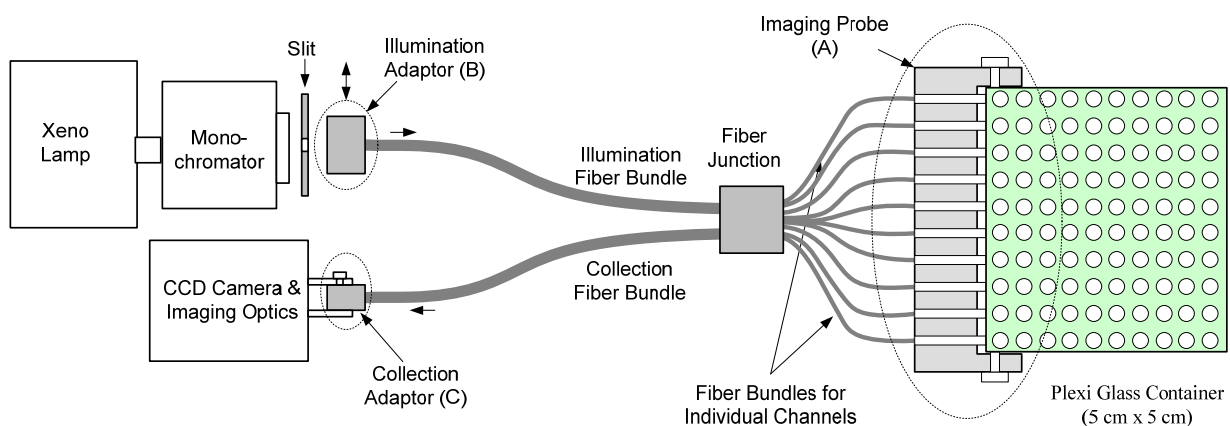

Figure 1. A schematic of the main components of the optical spectroscopic imaging system.

The majority of partial mastectomy specimens are smaller than 5 cm × 5 cm x 10 cm. Therefore, an imaging probe with a surface coverage of 5 cm × 5 cm will be designed. Within this area, the spatial resolution of the imaging system will be determined by the channel density of the optical probes, which is limited by cross-talk between adjacent probes due to tissue scattering. Photons from the illumination fibers of one channel may experience multiple scattering, propagate beyond the area within that channel, and may be collected by the collection fibers of another channel (most likely neighboring channels in the imaging probe), contributing to crosstalk. Crosstalk is determined by several factors, including channel spacing, the illumination and collection geometry and the tissue optical properties.

A series of Monte Carlo simulations (7-9) have been carried out to evaluate the crosstalk for the imaging probe. The configuration used for the cross-talk simulations is shown in Figure 2, which shows the channel of interest, channel 1 (CH 1), and the nearest neighbors (CH 2-9). Given the fact that diffuse reflectance attenuates exponentially in turbid medium, only the contributions to CH 1 from adjacent channels (CH 2-9) were taken into account. The simulations were carried out for the smallest absorption coefficient (μ_a=0.3 cm^{-1}) and a typical scattering coefficient (μ_s'=8.4 cm^{-1}) in the optical property range of breast tissue in the 400-600 nm range (5,6), i.e., for the case which would lead to the greatest cross-talk. Table 1 lists the calculated signal (contribution from CH1) to background (contribution from CH 2-9) for different channel spacings, D, for two distinct probe geometries. The first probe geometry has 19 fibers in the illumination core and 12 surrounding collection fibers (current single-channel probe) and the second probe geometry has 7 illumination and 4 collection fibers. All fibers have a 200 µm diameter and a 0.22 numerical aperture. The source-detector separation, r, is 0.735 mm (same as that of the current single-channel probe). From Table 1, it is obvious that a minimum channel spacing of 10 mm is required in order to achieve a signal to background of greater than 100 (1% cross-talk). With a channel spacing of 10 mm, a maximum of 25 channels can be built into a single imaging array to cover a 5 x 5 cm area.
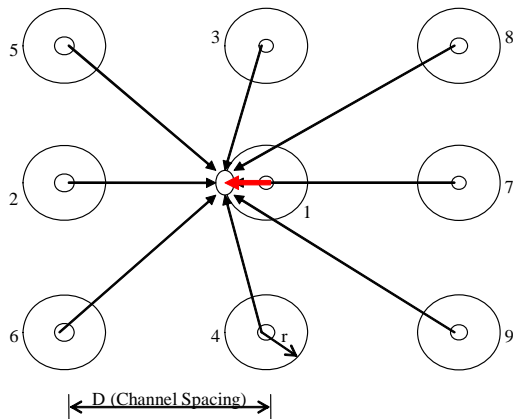

Figure 2. The configuration used for the cross-talk simulations Red arrow represents signal, black arrows represent crosstalk, D is the channel spacing, and r is the distance between the center of the illumination bundle and that of the collection fiber within the same channel.

Table 1. Signal (contribution from CH1) to background (contribution from CH 2-9) for different channel spacings (center-to-center distance between adjacent channels) for two different probe geometries.

Channel Spacing (mm)	3	4	5	6	8	10
19 illumination and 12 collection fibers	1.49	3.41	6.71	12.94	40.15	127.55
7 illumination and 4 collection fibers	1.45	3.18	6.51	12.26	40.04	114.35

All simulations were done using our current probe geometries which use 200 µm illumination fibers. However, constructing a probe with 7 illumination fibers is much more difficult than constructing a probe with a single larger illumination fiber. To simplify the fabrication process we chose to use a single 600 µm illumination fiber which is comparable to 7, 200 µm illumination fibers in diameter.

The number and/or size of the illumination fibers depend on the number of channels and the exit slit of the monochromator. Our current spectrometer has a monochromator with a slit height of 7 mm and a variable slit width. The relationship between the bandwidth and the slit width for this system is 2 nm/mm. For a 5 nm band pass the slit width would be set at 2.5 mm. When fibers with diameters of 600/660/710 µm (core/cladding/jacket) are used, the slit can accommodate an array of a maximum of 32 fibers. To not completely fill the slit, the total number of fibers will be reduced by approximately 25% to 25. For an imaging probe with 25 channels, the slit can accommodate 25, 600 µm illumination fibers.

The number of collection fibers for the imaging probe is based on the number of channels, number of pixels in the CCD, pixel size, magnification of the imaging optics, and fiber size. The calculated number of collection fibers is based on the current CCD chip in our spectrometer which is a 1024 x 256 thermoelectric open electrode CCD detector from HORIBA Jobin Yvon, Inc. in Edison, NJ. The total number of fibers that can be imaged on the CCD is based on the following equation:

$$f = \frac{(C_1 \times p - 4d) \cdot (C_2 \times p - 4d)}{\left(\left(\frac{\sqrt{n}+2}{\sqrt{n}} \right) \times \sqrt{M} \times d \right)^2} \tag{1}$$

Where, f is the total number of fibers, C_1 (1024)and C_2 (256) are the number of pixels in the vertical and horizontal direction, p is the pixel size (26 µm), n is the number of collection fibers per channel, M is the magnification of the system (1.1), and d is the fiber diameter (250 µm). The rule of thumb is to have at least a two fiber separation distance between each bundle of collection fibers to eliminate cross-talk on the CCD side (this has been verified experimentally on our CCD). Table 2 shows the maximum number of collection fibers that can be imaged on the CCD chip for the case where the number of collection fibers per channel is varied from 1 to 7.

Table 2. maximum number of collection fibers that can be imaged on the CCD for different values of n.

n	# fibers that can fit on the CCD	# of fibers needed for 100 channels
1	224	100
2	320	200
3	450	300
4	480	400
5	561	500
6	583	600
7	660	700

Our final goal is to construct an imaging probe with 100 channels. Therefore, 4 collection fibers were chosen to be certain that all of the fibers could be imaged on the CCD without any crosstalk between channels.

The objective of Task 2 was to build a two-channel imaging probe to test the conceptual design without having to build the entire 25 channel probe and to compare actual SNR and crosstalk results to simulated values. The two-channel probe has been built in our laboratory using 4, 200 µm collection fibers and 1, 600 µm illumination fiber in each channel. Aluminum adaptors for the illumination, collection, and probe tips were manufactured by our machine shop and used to secure the fibers in place with medical grade epoxy. A picture of the probe can be seen in Figure 3. The probe has been attached and aligned to our current system (Figure 4), where a number of experiments will be carried out to characterize the single channel probe. By visually inspecting the CCD there is no crosstalk between channels even at the highest reflectance.
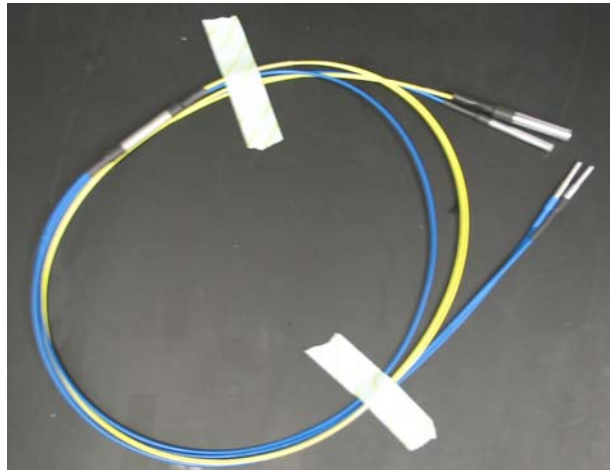

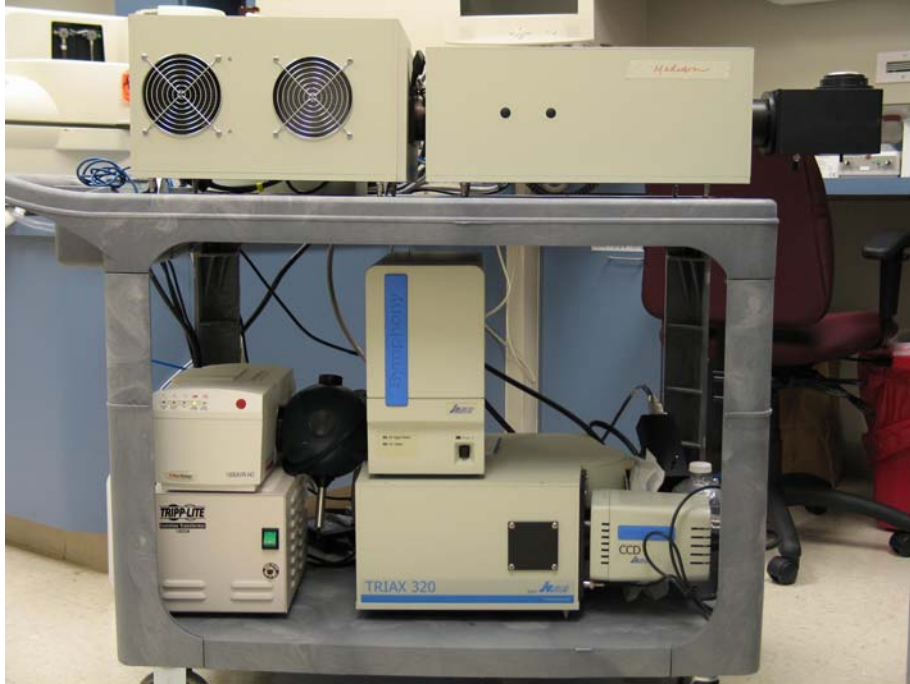*Figure 3. Photo of the completed two-channel fiber optic probe.*

Figure 4. Photo of our current clinical system.

Key Research accomplishments

- Simulated results for source-detector separations, signal-to-noise ratio, and cross-talk analysis have been completed.
- Built a two-channel imaging probe following the design of the final 25 channel probe
- A back-illuminated thermoelectrically cooled, 1024x1024 pixel UV/Visible CCD camera has been purchased from Princeton Instruments
- All necessary equipment for the imaging project has been ordered

Reportable Outcomes

Conclusions

The outcome of the proposed work is expected to result in a new optical diagnostic modality for breast cancer margin assessment at the time of tissue removal. Before the 25 channel imaging probe can be built and tested in a clinical setting, basic experiments must first be performed with the two-channel imaging probe. The two-channel experimental results will determine if any changes need to be made to the design of the 25 channel imaging probe. For example, if it turns out that the separation distance between two channels needs to be greater than 10mm, then it may be necessary to decrease the number of channels in the probe. The final spectroscopic imaging device will result in cosmetically-superior lumpectomies at the time of the first surgery, better margin assessment leading to reduced repeat surgeries and hence, reduced local

recurrence, emotional distress, and complications due to reduced number of surgeries and shorter recovery times for the patient.

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Appendix

1. Revised Statement of Work

Statement of Work

Near Infrared Spectroscopy for Improving Breast Core Needle Biopsy
Torre Michelle Bydlon, Investigator – Predoctoral Award Applicant

Task 1: Conceptual design of a spectrocopic imaging device (system and fiber optic probe) (Months 1 – 10)

    a. Design an imaging system (Months 1 – 10)
    b. Determine the appropriate number of channels for the imaging probe (Months 8 – 10)
        i. Dependent on cross-talk at the tissue surface, number of collection fibers that can be imaged on the CCD (charge coupled device), and number of illumination fibers that can be illuminated by the light source
    c. Determine the number of collection fibers that can be imaged on a variety of CCDs with different pixel sizes and calculate the throughput of the entire imaging system with the various CCDs (Months 8 – 10)
        i. Select a CCD to purchase for the imaging system
    d. SNR (signal to noise ratio) calculations to determine the appropriate spacing between each channel (Months 8 - 10)

Successful completion of this task will result in a design of an imaging system. The design for an imaging probe will also be complete and ready to build ourselves or by an industry partner.

Task 2: Build and test a two channel imaging probe (Months 11 – 14)

    a. Build an imaging probe with two channels where the channels have no specified separation distance (Months 11-12)
    b. Check that there is no crosstalk between signals on the CCD (Month 12)
    c. Using a liquid phantom, fix one channel in the center of the phantom and alter the separation distance between the fixed channel and the second channel to test the crosstalk between the two channels and compare to simulated results from Task 1 (Months 13 – 14)
    d. Repeatability of the probe (Months 13 – 14)
        i. A single channel will be placed in a phantom and 10 serial measurements will be made
        ii. Mean and standard deviation will be calculated for the 10 measurements at each wavelength
        iii. SNR will be calculated as the mean divided by the standard deviation
    e. Run an inverse Monte Carlo model, developed by our group, to determine how well the probe can extract known optical properties from tissue phantoms of hemoglobin and polystyrene spheres (Months 13 – 14)

Completing this task will allow us to determine how far apart each channel must be to avoid crosstalk on the tissue side of the probe. This will also allow us to

determine how efficiently the two channel probe is. If there is insufficient signal we may have to redesign the probe with greater separation distances between the illumination and collection fibers. We will also be able to tell if a self calibrating fiber is better than our current techniques of using an integrating sphere, puck, and water measurements to calibrate data. Completing the Monte Carlo inversions with two channels will help us to determine what difficulties we will face when trying to run the inversions with multiple channels and try to make the code more efficient at analyzing large amounts of data.

Task 3: Build a 25 channel imaging probe and system (Months 15 – 17)

    a. Build imaging probe
          i. Provide one of our industry partners, Romack or Polymicro, with our design specifications or build the probe ourselves
         ii. The probe specifications will be based on results that we find from the 2-channel probe tests
    b. The imaging system will consist of a monochromator, lens system for focusing and magnification of the signals from the probe, and a CCD.

Completion of this task will result in an optical spectral imaging system that will map out optical properties of turbid media and a probe to be used in tissue phantom studies and/or a clinical setting.

Task 4: Test imaging probe and system (Months 18 – 38)

    a. Complete a number of tissue phantom studies to characterize the system (Months 18 – 21)
    b. Analyze the phantom data by running an inverse Monte Carlo model to extract the optical properties of the phantoms and compute errors between the extracted values and the actual optical properties (Months 18 – 22)
    c. Complete an ex-vivo tissue analysis in the clinic with human subjects undergoing breast reduction surgery (Months 23 – 38)
          i. Collect optical spectral images from 50 partial mastectomy specimens
         ii. Extract the absorption and scattering coefficients from the optical spectra using an inverse Monte Carlo model
       iii. Compare extracted tissue properties to histopathology obtained for each margin imaged

Successful completion of this task will determine how accurate the imaging probe and system are at extracting optical properties of phantom tissue data as well as human tissue data.

*** Month 1 began September 2006